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# PATENT SPECIFICATION



Convention Date (United States): July 10, 1936.

500,354

Application Date (In United Kingdom): June 1, 1937. No. 15262/37.

Complete Specification Accepted: Feb. 1, 1939.

## COMPLETE SPECIFICATION

-9 MRZ. 1939

### Improvements in the Preservation of Desiccated Biologically Active Substances in Evacuated Containers

We, SHARP & DOHME, INCORPORATED, a Corporation organised and existing under the laws of the State of Maryland, United States of America, of 640, North Broad Street, Philadelphia, Pennsylvania, United States of America, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

The present invention relates to improvements in the preservation of biologically active substances in evacuated containers.

Biologically active substances such as sera, protein solutions, bacterial cultures, pharmaceutical and glandular substances, viruses and other labile biological substances are very sensitive to contamination or deterioration by air and moisture.

As now commonly distributed and marketed such substances are packaged and sealed in a liquid state. Such products are generally sold with a fixed expiration date after which they should not be used as they tend to deteriorate and to lose their biological properties. The marketing of the substances in this form often involves a serious economic loss owing to the loss of biological activity or potency between the time of manufacture and the time of use and the large proportion of the products which must be discarded at the end of the expiration date because of deterioration on storage.

It is known to preserve desiccated biologically active substances in containers under a high vacuum by means of a fused seal.

It has also been proposed in our prior British Patent Specification No. 450,147 to improve the stability and keeping properties of such biologically active substances by freezing such products and drying, that is removing water from, the frozen products under a high vacuum, both in bulk and in final containers. The said prior specification discloses the sealing of the desiccated product by means

of a perforable stopper under a high vacuum in the container in which it was prepared. The prior specification also envisages that in some instances air may be admitted into the container after the biologically active substance has been desiccated in it. In this case the prior specification proposes that the container is again evacuated, for example, by inserting a hollow needle connected to a high vacuum pump through the perforable stopper exhausting the container and then withdrawing the needle and permitting the stopper to hold the vacuum in the container.

Desiccated biologically active substances are very porous; it is important to seal and distribute them under a vacuum, both to prevent deterioration which might arise by contact with air or moisture, and to facilitate the dissolving of the material in water or other aqueous fluid when restoration of the substance to the liquid state is desired. When the material is maintained under a vacuum and water is introduced into the container before the vacuum is broken, the vacuum tends to pull the water into the pores and interstices of the material, insuring intimate contact of the water with the material. On the other hand, if air or other gas is introduced into the container before the water is introduced, the material tends to become air-bound, with the result that the water does not readily penetrate into it and the material does not dissolve rapidly.

When it is desired to restore to a liquid state desiccated material preserved under vacuum in a glass ampoule having a fused seal it is necessary to break the ampoule in order to introduce the solvent liquid. By this means, however, the vacuum is destroyed. In consequence the material becomes air-bound, it does not dissolve readily in the added liquid and must be shaken vigorously or allowed to remain in contact with the liquid for a considerable period of time before it dissolves.

When such a sealed ampoule is used, however, there is absolute assurance that

[Price 1/-]

the material is maintained under a high vacuum free from contact with air or moisture or contaminating matter, regardless of how long a period of time it may be stored.

On the other hand, when the desiccated material is sealed in a container having a perforable closure, such as a rubber stopper, water or other liquid may be introduced into the container, as by means of a hypodermic needle, to enable the material to be restored to a liquid state without destroying the vacuum. Under such conditions, the material dissolves rapidly and readily, because of the intimate contact between the liquid and the desiccated material.

The object of the present invention is to provide an evacuated container enclosing a desiccated biologically active substance such that the advantages of an ampoule or container sealed by fusion and those of an ampoule closed by a perforable stopper are combined.

The invention accordingly comprises a sealed evacuated container enclosing a desiccated biologically active substance and having a neck in which is disposed a perforable stopper, characterised in that a portion of the neck extends beyond the stopper and is sealed by fusion therebeyond so as to enclose the stopper within it. Preferably, the desiccated biologically active substance within the container has a porous, highly capillary structure which is of such shape as to indicate that it was formed within the container.

The portion of the neck between the stopper and the fused seal is preferably evacuated.

Such containers render it possible to effect the introduction of water thereinto without materially disturbing the vacuum, while during storage and transport the contents of the container and the vacuum are protected by the fused seal. The perforable stopper is also protected from deterioration and exposure as it is sealed wholly within the neck of the container.

A further feature of the invention resides in the method of sealing a desiccated biologically active substance in an evacuated container having a neck, after the application of vacuum through the neck to desiccate the substance, which comprises releasing the vacuum after desiccation has been effected, then introducing a perforable resilient stopper into the neck of the container so far that the neck extends beyond the stopper, re-evacuating the container through the stopper, and thereafter sealing off the neck by fusion beyond the stopper so as

to enclose the stopper within it.

The invention also includes a sealed evacuated container enclosing a desiccated biologically active substance when produced in accordance with the said method.

The invention will now be described with reference to the accompanying drawings, in which:—

Figure 1 is a sectional view of a glass container after the desiccated material has been produced therein,

Figure 2 is a sectional view of the container of Figure 1 after a perforable rubber stopper has been introduced into its neck, showing the means by which a vacuum is produced in the container;

Figure 3 is a view showing the final container after sealing; and

Figure 4 is a view showing the container after the neck containing the perforable stopper is broken off and showing the means by which water may be introduced.

In Figure 1, 10 represents a substantially cylindrical vial or container of suitable size intended for the marketing or distribution of a desiccated serum or other biologically active substance. Within the container is located a desiccated biologically active substance 11, which has been produced therein by introducing into the container a biologically active substance in liquid form freezing it rapidly preferably while the container is in a more-or-less horizontal position by immersing the container in a refrigerant maintained at a very low temperature, e.g.  $-70^{\circ}\text{C}$ ., attaching the container to a vacuum manifold and subliming or evaporating the ice therefrom with the aid of a high vacuum.

The heat absorbed during the sublimation or vaporization is sufficient to maintain the material in a frozen state despite the flow of heat into the material from the outside. In order to maintain the sublimation or vaporization at a sufficient rapid rate to prevent the melting of the material, it is necessary to provide an adequately large passageway for the vapors, and to avoid the use of vapor passages of too small a lumen, or with too many constrictions. The distribution of the material lengthwise of the container assists in securing a maximum exposed surface for sublimation. The desiccated material so produced is maintained as a formed porous mass, having the shape and volume of the frozen material from which it is produced, without change in its physical structure after desiccation, and having an immense network of capillaries or pores. The container 10, is provided with a relatively

long neck 12, of sufficient diameter to provide for the free flow of vapors from the interior of the container and the neck is tapered over the portion 13 near the top of the container body to fit a rubber stopper as described hereinafter. With regard to the size of the container this may be varied almost at will. Its capacity will be determined by the amount of material which it is intended to contain preferably being such as to contain one or more unit portions of the material. Thus the capacity of the container may vary from a fraction of a cubic centimeter to 50 or 100 or more cubic centimeters. The volume of the container, however, must be somewhat more than twice as great as the volume of the material intended to be processed therein, in order to provide adequate surface for the sublimation or evaporation of water therefrom. Thus if unconcentrated material is processed in the container, the container must have a volume of about twice the volume of the final restored product, whereas if concentrated material, such as material which has been concentrated to about one-half volume in a suitable manner, is processed within the container, the container may have an amount of desiccated material within it which on restoration to its normal liquid condition about fills the container. The neck of the vial, even where tapered, must be of sufficient size to permit the free flow of water vapor during the desiccation process.

After the desiccated material has been produced within the container 10, the container is removed from the vacuum apparatus and a perforable stopper, e.g., a rubber stopper, such as the stopper 14 shown in Figure 2 is introduced into the neck 12 and forced down into engagement with the tapered portion 13 of the neck so as to form a tight joint therewith. The stopper 14 is advantageously provided with a passage 15 part way through it to facilitate the passage of a needle. After the stopper is forced into position, a hollow needle 16, such as a hypodermic needle, which is joined by connection 17 to a suitable vacuum device is passed through the perforable stopper. The interior of the container is thus exhausted, and after a vacuum is produced within the container, the needle is withdrawn. The stopper 14 then serves to hold the vacuum within the container. The glass neck 12 of the container which extends considerably beyond the rubber stopper is then flame-sealed, to produce a container such as illustrated in Figure 3. In order to seal the neck of the container, it is advantage-

lous to heat a portion of the neck by means of a broad flame and draw it to capillary dimensions and then to connect the neck again to a vacuum pump or manifold and seal it by means of a flame. By following this procedure, a seal such as shown at 18 is obtained, with the portion of the neck between the stopper 14 and the seal 18 evacuated as well as the interior of the container 10.

With special precautions, or with the use of certain types of glass, such as the glass known under the Registered Trade Mark "Pyrex", the neck 12 may be sealed while a vacuum is maintained therein, without first drawing a portion of it to a fine tube, but it is advantageous to draw a portion to a fine tube before sealing, as this simplifies the production of a proper seal, and avoids the difficulties encountered in sealing a relatively large tube under a high vacuum.

Flame-sealing the neck of the container while the neck is connected to a vacuum insures the presence of a vacuum in the sealed neck between the rubber stopper and the glass seal as well as in the container proper. This is a particularly advantageous method of sealing the containers, and is the method which we prefer to use. Nevertheless, the invention is not limited to this procedure as the glass seal may be made without evacuating the neck during the sealing operation. This alternative procedure may be used particularly with those containers in which the volume of the container is large relatively to the neck, i.e. is many times as great as the volume of the neck, or that portion of the neck between the rubber stopper and the glass seal. Even when the neck is flame-sealed without first producing a vacuum within the neck the air within is greatly attenuated by the heat required to seal the glass. The air is also free from any appreciable amount of moisture so that there is but little air present above the rubber stopper to penetrate past the stopper into the container, and almost no moisture. Hence, even if some air should enter the container, the amount which can enter is small, and the amount of moisture which can enter is almost infinitesimal. It will, therefore, be seen that the vacuum within the container (particularly where the container is relatively large) cannot be impaired to any great degree, and sufficient air to interfere with the proper solution of the material on the introduction of water cannot enter the container.

When it is desired to restore the material, it is simply necessary to break off the upper portion of the neck 12 (this 130

portion of the neck being etched or scratched a little below the top of the rubber stopper as at 19, Figure 3, to facilitate the breaking off of the upper portion of the neck-producing a container having a rubber stopper as shown in Figure 4) and to introduce the needle 20 of a hypodermic syringe, or a needle suitably connected to a vial containing water. A predetermined amount of water is then permitted to flow through the needle into the container, without permitting the entrance of air. The desiccated material is thoroughly wetted and penetrated by the water aided by the action of the vacuum within the container and air is introduced into the container to force the water into intimate contact with the inner portions of the desiccated material. The restored liquid material may then be withdrawn by means of the syringe.

It will thus be seen that by means of the present invention, desiccated biologically active substances may be preserved in a final container having an all-glass seal which ensures the maintenance of the vacuum under which the desiccated material is maintained and prevents moisture or other contaminating substances entering the container, and also having an interior perforable seal, which permits the introduction of water or other liquid by means of a needle or the like without destroying the vacuum within the container, which is adequate to maintain the vacuum within the container for such periods of time as may be required in the restoration of the material to a liquid state, or in the production of the glass seal.

No claim is made herein to anything claimed in our co-pending Patent Application No. 14926/37 (Serial No. 500,255).

Having now particularly described and ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is:—

1. A sealed evacuated container enclosing a desiccated biologically active substance and having a neck in which is disposed a perforable stopper, characterised in that a portion of the neck extends beyond the stopper and is sealed by fusion therebeyond so as to enclose the stopper within it.

2. A sealed evacuated container en-

closing a desiccated biologically active substance and having a neck in which is disposed a perforable stopper, characterised in that the desiccated biologically active substance has a porous, highly capillary structure, which is of such shape as to indicate that it was formed within the container and that a portion of the neck extends beyond the stopper and is sealed by fusion therebeyond so as to enclose the stopper within it.

3. An evacuated container as claimed in claim 1 or claim 2, wherein the portion of the neck between the stopper and the fused seal is evacuated.

4. An evacuated container as claimed in claim 1 or claim 2 or claim 3, wherein the neck tapers towards the container and the stopper is located in tight engagement with the tapered portion.

5. The method of sealing a desiccated biologically active substance in an evacuated container having a neck, after the application of vacuum through the neck to desiccate the substance, which comprises releasing the vacuum after desiccation has been effected, then introducing a perforable resilient stopper into the neck of the container so far that the neck extends beyond the stopper, re-evacuating the container through the stopper and thereafter sealing off the neck by fusion beyond the stopper so as to enclose the stopper within it.

6. A method as claimed in claim 5, characterised by evacuating the neck portion beyond the stopper and maintaining the vacuum therein while sealing by fusion is being effected.

7. The method of producing biologically active substances sealed under vacuum in a desiccated condition substantially as described with reference to the drawings.

8. A sealed evacuated container enclosing a desiccated biologically active substance when produced in accordance with claim 5 or claim 6.

9. A sealed evacuated container enclosing a desiccated biologically active substance substantially as described with reference to Figure 3 of the accompanying drawings.

Dated this 1st day of June, 1937.  
SHARP & DOHME INCORPORATED.  
Per: Boulton, Wade & Tennant.  
111/112, Hatton Garden, London, E.C.1.  
Chartered Patent Agents.

Fig. 1.

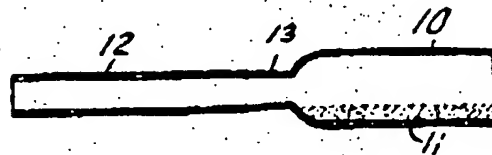


Fig. 2.

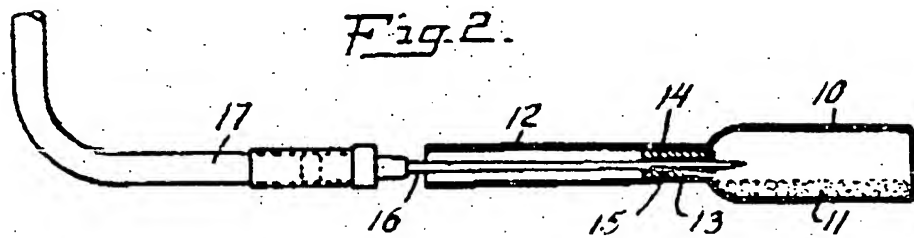


Fig. 3.

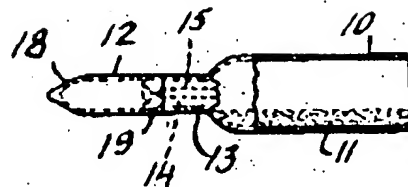
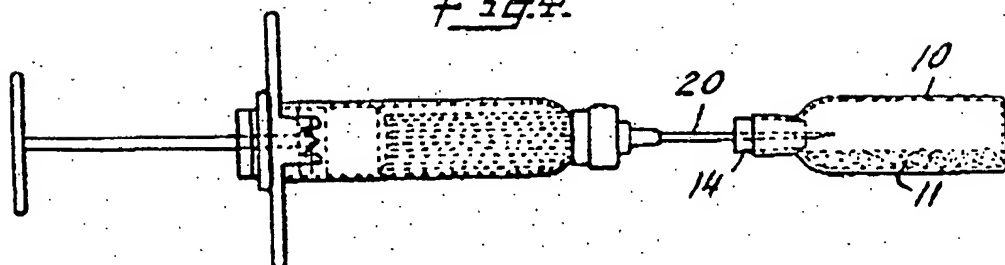


Fig. 4.



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